REMARKS UNDER 37 CFR § 1.111

Formal Matters

Claims 1, 4-8, and 11 are pending after entry of the amendments set forth herein.

Claims 1-11 were examined. Claims 1-11 were rejected.

Please replace claims 1 and 5 with the clean version provided above.

Claims 2-3 and 9-10 have been canceled. The Applicants note that these claims are canceled without prejudice to renewal and solely to expedite prosecution. The Applicants do not intend to abandon the subject matter encompassed therein. The Applicants expressly reserve the right to file one or more subsequent applications directed to the cancelled subject matter.

Claims 1 and 5 have been amended to include a positive process step and to specify that the agent reduces the expression of apoE in the host. Support for this amendment is found throughout the specification, and in particular at: page 14, line 12.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the amendments and remarks made herein.

No new matter has been added by the above amendments. Accordingly, their entry by the Examiner is respectfully requested.

Objection to the Specification

The specification has been objected to because the upper margins are not large enough. Upon indication of allowable subject matter, the Applicants will provide a substitute specification with appropriate margins.

Rejection under 35 U.S.C. §112, first paragraph

The rejection of Claims 1-11 under 35 U.S.C. §112, first paragraph, has been maintained for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

The Applicants respectfully submit that one of ordinary skill in the art would recognize that the Applicants invented that which is claimed. The present invention reflects the Applicants novel discovery that overexpression and accumulation of apoE stimulates VLDL production and impairs VLDL lipolysis. Plasma VLDL-triglyceride levels are determined by three metabolic parameters: 1) hepatic VLDL-triglyceride production rate, 2) plasma clearance rate of the VLDL-triglyceride, and 3) hydrolysis rate of the VLDL-triglyceride to convert VLDL to LDL. Altering any of these three will change plasma VLDL-triglyceride levels. Too much apoE affects two of these parameters by stimulating VLDL-triglyceride production in the liver and impairing VLDL hydrolysis, resulting in an accumulation of VLDL in the host.

In the instant application, the Applicants have shown that VLDL production is positively correlated with plasma apoE levels by creating knockout and transgenic mice expressing different levels of apoE (see Fig. 2). For example, the Applicants crossbred male transgenic mice expressing low or high levels of human apoE with female apoE knockout mice to generate apoE transgenic mice without endogenous mouse apoE. The results of this experiment are depicted in Fig. 5, which clearly shows statistically significant increasing VLDL production rate for increasing human apoE expression, from nontransgenic to low producing to high producing transgenic mice. It has also been shown that apoE deficient mice (homozygous knockout) have a decrease in hepatic VLDL production (see Kuipers et al., J. Clin. Invest. (1997) 100:2915-22 (copy enclosed)). Note, however, that these homozygous apoE knockout mice also have increased plasma levels of VLDL (see page 2917, Table 1) because complete absence of apoE impairs the clearance of apoE from the plasma.

The Applicants have also shown that human subjects with hyperlipidemia have elevated levels of apoE and VLDL compared to normolipidemic subjects (see, page 33, lines 8-12; Figs. 4 and 7; and Huff et al., page 223, table 1). In addition, the Applicants have shown that increasing apoE expression results in an increase in VLDL production and Kuipers et al. has shown that eliminating apoE expression results in a decrease in VLDL production. Furthermore, plasma or VLDL apoE levels correlate negatively with VLDL lipolysis (Fig. 2D) meaning that as apoE level increases, VLDL lipolysis is impaired with the result that VLDL levels increase. The Applicants have also shown experimentally

that apoE impairs this LPL-mediated lipolysis of VLDL (see page 33, lines 21-27). The combination of these data and others contained in the application indicate that overexpression and accumulation of apoE causes hyperlipidemia by stimulating VLDL production and impairing VLDL lipolysis. Since, VLDL production rate is determined by the level of apoE expression and hyperlipidemic subjects have elevated levels of both VLDL and apoE, it follows that by decreasing apoE expression, one will also decrease the plasma level of VLDL.

The specification provides ample guidance such that one skilled in the art could use the specification, coupled with that which is known in the art, to practice the claimed invention. The Applicants detail several methods of decreasing expression of apoE on page 14, line 12 through page 16, line 16. Once a skilled artisan has identified a gene target, reducing its expression is well within that artisan's skill. For example, since the nucleotide sequence of apoE is known, one of skill in the art would fully expect to be able to use antisense technology to reduce apoE expression. Furthermore, researchers, such as Charpentier et al. (Biochemistry (2000) 39:16084-91 (copy enclosed)) have already demonstrated that antisense technology can be used to reduce apoE expression.

In addition, the Applicants have disclosed methods of screening agents for effectiveness, and have even provided animal models, in the specification at, for example, page 23, line 11 through page 26, line 26, and throughout the Experimental section. Such disclosure includes methods for detecting and quantifying apoE, VLDL, and triglycerides. Thus, many different agents are described in the specification and the specification also provides a method for screening agents for their ability to at least reduce the expression of apoE.

All that is necessary to fulfill the written description requirement is that one of skill in the art recognize that the Applicants invented what is claimed. MPEP §2163.02. The Applicants have disclosed the novel discovery that overexpression and accumulation of apoE stimulates VLDL triglyceride production and impairs VLDL lipolysis, have provided a list of agents suitable for use in the present invention, have provided references that demonstrate the use of such agents, and have provided methods and animal models for determining which agents are useful in the claimed methods. The Applicants' discovery, coupled with the disclosure in the specification and that which is known in the art, would convey to the skilled artisan that the Applicants were in possession of the claimed invention. Accordingly, the Applicants respectfully request that this rejection of Claims 1-11 under 35 U.S.C. §112, first paragraph, be withdrawn.

The rejection of Claims 1-11 under 35 U.S.C. §112, first paragraph, has been maintained for the asserted reason that the claims contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which they pertain, or with which they are most nearly connected, to make and/or use the invention. The Examiner contends that the amount of experimentation that would be required to practice the claimed invention with a reasonable expectation of success is unwarranted because the specification does not teach which, if any, of the many thousands of putative agents that are encompassed by the claims can be used effectively to reduce the level of apoE and VLDL in a patient. The Examiner also argues that the success of using antisense technology is very limited, there is no factual evidence that a reduction in the level of plasma active apoE can effectively reduce the production of VLDL in a patient, and there is no actual evidence that expression and accumulation of apoE in mice or humans is causative of hyperlipidemia, per se.

As amended, the claims are directed to a method for reducing the plasma level of VLDL in a host, and a method of treating a host suffering from a disease condition associated with elevated plasma level of VLDL, by administering to the host an effective amount of an agent which at least reduces the expression of apoE in the host by an amount sufficient to reduce VLDL level or production. The Applicants respectfully submit that the specification and the amended claims, coupled with the information known in the art, would enable one of skill in the art to use the invention without undue experimentation.

As the Applicants noted previously, the test of enablement is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine. Once a skilled artisan has identified a gene target, reducing its expression is well within that artisan's skill with no more than routine experimentation. One common method of reducing expression of a gene is by using antisense technology. The Examiner contends that such technology requires undue experimentation and cites James et al. and Roush et al. in support.

The Applicants note that these two references were published in 1991 and 1997, respectively. Since that time, the use of antisense has made great advances and its use to reduce gene expression is routine, as evidenced by the literature. For example, there are now at least four books which detail techniques, protocols, and strategies for use of antisense to modify gene expression, including those by Stein and Krieg (Applied Antisense Oligonucleotide Technology, Wiley-Liss, 1998); Lichtenstein and Nellen (Antisense Technology: A Practical Approach, Oxford University Press, 1997); Leslie et al. (Antisense Technology in the Central Nervous System, Oxford University Press, 1999); and

overexpression and accumulation of apoE causes hyperlipidemia; however, it is important to note that the claims only require the Applicants to demonstrate that apoE *affects* VLDL production and also the plasma level of VLDL, as they have done. The Applicants' invention is a method for reducing plasma VLDL level by reducing apoE expression. All that matters is that reducing expression of apoE also reduces VLDL production and plasma level of VLDL, which the Applicants have thoroughly demonstrated.

Thus, potential agents, such as antisense oligonucleotides, for reducing expression of apoE are described in the specification. Furthermore, the specification provides methods of screening for agents useful in the methods of the present invention and it would be well within the ability of the ordinarily skilled artisan to use the specification, coupled with that which is known in the art, to practice the subject invention without undue experimentation. Accordingly, the Applicants respectfully request that this rejection of Claims 1-11 under 35 U.S.C. §112, first paragraph, be withdrawn.

Rejection under 35 U.S.C. §112, second paragraph

The rejection of Claims 1-11 has been maintained under 35 U.S.C. §112, second paragraph, as assertedly indefinite for failing to particularly point out and distinctly claim the subject matter that the Applicants regard as their invention. The Office Action contends that claims 1 and 5 are indefinite because there is no positive process step that clearly relates back to the preamble of the claims.

While disagreeing with this rejection, and solely to expedite prosecution, the Applicants have amended to claims 1 and 5 to add the Examiner's suggested language to the claims. These amendments obviate this rejection.

Rejection under 35 U.S.C. §102(b)

The rejection of Claims 1-11 has been maintained under 35 U.S.C. §102(b) as allegedly anticipated by Ditschuneit et al., as evidence by Pedreno et al. and Durrington et al. The previous Office Action asserted that Ditschuneit et al. teaches a method of treating female patients with hyperlipoproteinaemia type IV with gemfibrozil and that the mechanism by which an agent acts to treat a disease is an inherent property of that agent. The Office Action then pointed to Pedreno et al. and Durrington et al., claiming they teach that gemfibrozil causes a reduction in levels of triglyceride, VLDL and apoE in a patient.

As amended, the claims are directed to a method of reducing plasma VLDL level in a host, and treating a host suffering from a disease condition associated with elevated plasma level of VLDL, by

"administering to said host an effective amount of an agent which at least reduces the amount of plasma active apoE in said host by reducing the expression of apoE by an amount sufficient to reduce VLDL production in said host to reduce the plasma level of VLDL in said host." Thus, the claims clearly require that the reduction in plasma VLDL is a direct result of reducing the apoE expression. As explained previously, the mechanism of action of gemfibrozil is through increasing LDL receptor expression, for which apoE is a ligand. Since apoE is a component of VLDL, an increase in LDL receptor will result in enhanced clearance of plasma VLDL and, because apoE is a component of the VLDL particles, apoE levels will also be reduced when VLDL clearance is increased. Nothing in the references teach that gemfibrozil reduces expression of apoE or that reducing apoE expression will reduce VLDL production and consequently reduce the plasma VLDL level.

Thus, the claimed invention and the mechanism of gemfibrozil are not the same. Accordingly, Ditschuneit et al., as evidenced by Pedreno et al. and Durrington et al., does not anticipate Claims 1-11 and this rejection under 35 U.S.C. §102(b) should be withdrawn.

The rejection of Claims 1, 3-8, and 10-11 has been maintained under 35 U.S.C. §102(b) as allegedly anticipated by Yoshino et al. The previous Office Action asserted that Yoshino *et al.* teaches that treating patients with pravastatin results in a significant decrease in the levels of triglyceride, VLDL, and apoE in the plasma of the patients.

As pointed out above, the claims have been amended such that they are directed to a method of reducing plasma VLDL level in a host, and treating a host suffering from a disease condition associated with elevated plasma level of VLDL, by administering an agent which at least reduces the expression of apoE in the host to reduce VLDL production, thereby reducing the plasma VLDL level. As discussed previously, there is no evidence that pravastatin acts to decrease plasma VLDL level by reducing expression of apoE, while there is evidence that it acts through a different mechanism in that it is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This inhibition of HMG-CoA reductase leads to upregulation of the LDL receptor, which, in turn, leads to enhanced clearance of plasma VLDL. Since apoE is a component of the VLDL particles, apoE levels will also be reduced when VLDL is cleared; however, this decrease in apoE is not the result of decreased expression as the claims require. Nothing in the references teach that pravastatin reduces expression of apoE or that reducing apoE expression will reduce VLDL production and consequently reduce the plasma VLDL level.

Thus, there is nothing in Yoshino *et al.* to suggest that pravastatin decreases plasma VLDL level by reducing apoE expression. Accordingly, this rejection of Claims 1, 3-8, and 10-11 under 35 U.S.C. §102(b) should be withdrawn.

The rejection of Claims 1, 3-8, and 10-11 has been maintained under 35 U.S.C. §102(b) as allegedly anticipated by Connor et al. The previous Office Action contended that Connor et al. teaches a dramatic reduction in plasma triglycerides resulting from treatment with dietary n-3 fatty acids, as well as a decrease in the levels of VLDL and apoE.

Nothing in the cited reference teaches that apoE is a target for reducing the plasma level of VLDL or that reduction of apoE expression will cause a reduction in VLDL production and thereby also reduce plasma VLDL, while there is evidence that n-3 fatty acids act through a different mechanism. As noted previously, if the cited agent decreased VLDL production by decreasing apoE, one of skill in the art would expect that it would have other effects opposite to that of an overexpression of apoE. Huang et al. (cited in previous Response) teaches that increased apoE results in normal or decreased LDL levels by impairing VLDL lipolysis; however, Connor et al. states that dietary n-3 fatty acids caused a reduction in LDL. This is the opposite effect on LDL level that would be expected if n-3 fatty acids acted by decreasing apoE expression and would lead one of skill in the art to believe that the mechanism of action is not through apoE expression. Connor et al. further states that dietary n-3 fatty acids reduce synthesis of triglyceride and VLDL in the liver and shorten turnover of VLDL in the plasma. This statement is supported by both Harris (J. Lipid Res. (1989) 30:785-807 (abstract enclosed)) and Hebbachi et al. (Biochem. J. (1997) 325:711-9 (abstract enclosed)). Furthermore, Anil et al. (Biochem. Mol. Biol. Int. (1997) 43:1071-80 (abstract enclosed)) report that the effect of n-3 fatty acids on hepatic VLDL production is mediated through prostaglandins. Nothing in the references teach that dietary n-3 fatty acids reduce expression of apoE or that reducing apoE expression will reduce VLDL production and consequently reduce the plasma VLDL level.

Thus, there is nothing in Connor *et al.* to suggest that dietary n-3 fatty acids decrease VLDL production by reducing apoE expression. Accordingly, this rejection of Claims 1, 3-8, and 10-11 under 35 U.S.C. §102(b) should be withdrawn.

Conclusion

In view of the above remarks, this application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issue.

If the Examiner finds that a Telephone Conference would expedite prosecution of this application, he is invited to contact the undersigned (650) 327-3400.

In the event that the transmittal letter is separated from this document and the Patent Office determines that extensions or other relief is required and/or fees are due applicants, the Applicant petitions for any required relief, including extensions of time, and authorize the Commissioner to charge our Deposit Account No. 50-0815, Order Number UCAL121, for any fees due in connection with the filing of this document. The Patent Office is not authorized to charge issue fees to our Deposit Account.

Respectfully submitted,
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Enclosures:

- Exhibit A
- Kuipers et al., J. Clin. Invest. (1997) 100:2915-22
- Charpentier et al., Biochemistry (2000) 39:16084-91
- Sze et al., Neurochem. Int. (2001) 39:319-327
- Finegold et al., Mol. Brain Res. (2001) 90:17-25
- Tamm et al., Lancet (2001) 358:489-497
- Agrawal and Kandimalla, Mol. Med. Today (2000) 6:72-81
- Harris, J. Lipid Res. (1989) 30:785-807
- Hebbachi et al., Biochem. J. (1997) 325:711-9
- Anil et al., Biochem. Mol. Biol. Int. (1997) 43:1071-80

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 2-3 and 9-10 have been canceled.

Claims 1 and 5 have been amended as follows.

1. (Twice Amended) A method for reducing the plasma level of VLDL in a host, said method comprising:

administering to said host an effective amount of an agent which at least reduces the amount of plasma active apoE in said host by reducing the expression of apoE by an amount sufficient to reduce VLDL production in said host to reduce the plasma level of VLDL in said host, whereby the plasma level of VLDL in said host is reduced.

5. (Twice Amended) A method of treating a host suffering from a disease condition associated with elevated plasma levels of VLDL, said method comprising:

administering to said host an effective amount of an agent that at least reduces the plasma amount of active apoE in said host by reducing the expression of apoE by an amount sufficient to reduce VLDL production to treat said disease condition, whereby said host is treated.